



SEQ\_LISTING\_11302009  
SEQUENCE LISTING

<110> Korea Research Institute of Bioscience and Biotechnology  
<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase  
<130> 26666U  
<140> 10/527,438  
<141> 2005-03-11  
<150> PCT/KR03/01820  
<151> 2003-09-04  
<150> KR 2002-55575  
<151> 2002-09-13  
<160> 19  
<170> PatentIn version 3.5  
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<213> Artificial Sequence

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<220>  
 <223> GPD-err primer  
 <400> 4  
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 <212> DNA  
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<220>  
 <223> T-0 primer  
 <400> 5  
 tgcagttgaa cacaaccac 19

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 <211> 1023  
 <212> DNA  
 <213> Candida antarctica

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 gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc 300  
 cagggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360  
 aacaagcttc ccgtgctcac ctggtcccgag ggtggtctgg ttgcacagtg ggggtctgacc 420  
 ttcttcccca gtatcagggtc caagggtgat cgacttatgg cctttgcgcc cgactacaag 480  
 ggcaccgtcc tcgccggccc tctcgatgca ctgcgggta gtgcaccctc cgtatggcag 540  
 caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtggtct gaccagatc 600  
 gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660  
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 gggccgctgt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780  
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 gactgcaacc ctcttcccgcc caatgatctg actcccagac aaaaggctgc cgcgggctgcg 900  
 ctcccggcgc cggcggtgac agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960  
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ggcaccacag gtccacagtc gttcgactcg aactggatcc ccctctctgc gcagctgggt 180  
tacacaccct gctggatctc acccccgcgc ttcattgtca acgacacca ggtcaacacg 240  
gagtacatgg tcaacgccat caccacgctc tacgctgggt cgggcaacaa caagcttccc 300  
gtgctcacct ggtcccaggg tggctctggt gcacagtggg gtctgacctt cttccccagt 360  
atcaggcca aggtcgatcg acttatggcc tttgcgccc actacaaggg caccgtcctc 420  
gccggccctc tcgatgcact cgcggttagt gcaccctccg tatggcagca aaccaccggt 480  
tcggcactca ctaccgcact ccgaaacgca ggtggtctga cccagatcgt gccaccacc 540  
aacctctact cggcgaccga cgagatcggt cagcctcagg tgtccaactc gccactcgac 600  
tcattcctacc ttttcaacgg aaagaacgct caggcacagg ctgtgtgtgg gccgcagttc 660  
gtcatcgacc atgcaggctc gctcacctcg cagttctcct acgtcgtcgg tcgatccgcc 720  
ctgcgtcca ccacgggcca ggctcgtagt gcggactatg gcattacgga ctgcaaccct 780  
cttcccgcga atgatctgac tcccagagca aaggctcgcc cggctgcgct cccggcgccg 840  
gcggctgcag ccatcgtggc gggccaag cagaactgcg agcccgacct catgccctac 900  
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<210> 8  
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<212> DNA  
<213> Candida antarctica

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gatgcgggtc tgacctgcca ggggtgcttc ccattctcgg tctccaaacc catccttctc 180  
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gcgcagctgg gttacacacc ctgctggatc tcaccccgcc cgttcattgct caacgacacc 300  
caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360  
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggctctgacc 420  
ttcttcccca gtatcaggct caaggctgat cgacttatgg ctttgcgcc cgactacaag 480  
ggcaccgtcc tcgcccggcc tctcgatgca ctgcgggta gtgcaccctc cgtatggcag 540  
caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtggtct gaccagatc 600

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|                                                                   |      |
|-------------------------------------------------------------------|------|
| gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac | 660  |
| tcgccactcg actcatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt | 720  |
| gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc | 780  |
| ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacg | 840  |
| gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggctcg cgcggtcg   | 900  |
| ctcctggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac | 960  |
| ctcatgccct acgcccggcc ctttgcagta ggcaaaagga cctgctccgg catcgtcacc | 1020 |
| ccc                                                               | 1023 |

<210> 9  
 <211> 319  
 <212> PRT  
 <213> Candida antarctica

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Ser | Gly | Ser | Asp | Pro | Ala | Phe | Ser | Gln | Pro | Lys | Ser | Val | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ala | Gly | Leu | Thr | Cys | Gln | Gly | Ala | Ser | Pro | Ser | Ser | Val | Ser | Lys |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Ile | Leu | Leu | Val | Pro | Gly | Thr | Gly | Thr | Thr | Gly | Pro | Gln | Ser | Phe |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ser | Asn | Trp | Ile | Pro | Leu | Ser | Ala | Gln | Leu | Gly | Tyr | Thr | Pro | Cys |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Ile | Ser | Pro | Pro | Pro | Phe | Met | Leu | Asn | Asp | Thr | Gln | Val | Asn | Thr |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Tyr | Met | Val | Asn | Ala | Ile | Thr | Thr | Leu | Tyr | Ala | Gly | Ser | Gly | Asn |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Lys | Leu | Pro | Val | Leu | Thr | Trp | Ser | Gln | Gly | Gly | Leu | Val | Ala | Gln |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Gly | Leu | Thr | Phe | Phe | Pro | Ser | Ile | Arg | Ser | Lys | Val | Asp | Arg | Leu |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Phe | Ala | Pro | Asp | Tyr | Lys | Gly | Thr | Val | Leu | Ala | Gly | Pro | Leu |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ala | Leu | Ala | Val | Ser | Ala | Pro | Ser | Val | Trp | Gln | Gln | Thr | Thr | Gly |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

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Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile  
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro  
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys  
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His  
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
260 265 270

Ala Ala Ala Ala Leu Pro Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro Gly Ser  
305 310 315

<210> 10  
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<212> PRT  
<213> Candida antarctica

<400> 10

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu  
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Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys  
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Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe  
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys  
50 55 60

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Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr  
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Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn  
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln  
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu  
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu  
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly  
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile  
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro  
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys  
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Gln Phe Val Ile Asp His  
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
260 265 270

Ala Ala Ala Ala Leu Pro Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro Gly Ser

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305

310

<210> 11  
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<212> PRT  
<213> Candida antarctica

<400> 11

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20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe  
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys  
50 55 60

Trp Ile Ser Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr  
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn  
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln  
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu  
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu  
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly  
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile  
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro  
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys  
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Gln Phe Val Ile Asp His  
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220

210

215

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro  
305 310 315

<210> 12  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> CALB primer 4

<400> 12  
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26

<210> 13  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> a-amylase secretion signal

<400> 13

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Ala Pro Ala Leu Ala  
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<210> 14  
<211> 317  
<212> PRT  
<213> Candida antarctica

<400> 14



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 20 25 30  
 Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe  
 35 40 45  
 Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys  
 50 55 60  
 Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr  
 65 70 75 80  
 Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn  
 85 90 95  
 Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln  
 100 105 110  
 Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu  
 115 120 125  
 Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu  
 130 135 140  
 Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly  
 145 150 155 160  
 Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile  
 165 170 175  
 Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro  
 180 185 190  
 Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys  
 195 200 205  
 Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His  
 210 215 220  
 Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
 225 230 235 240  
 Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
 245 250 255

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Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro  
305 310 315

<210> 15  
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<212> DNA  
<213> Artificial Sequence

<220>  
<223> LQ53 primer

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28

<210> 16  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> LQ35 primer

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30

<210> 17  
<211> 30  
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<400> 17  
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30

<210> 18  
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<212> DNA  
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<223> LP35 primer

<400> 18

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<210> 19  
 <211> 343  
 <212> PRT  
 <213> Candida antarctica

<400> 19

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
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Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
 35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
 50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
 65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
 85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
 100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
 115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
 130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
 145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
 165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
 180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
 195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
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210

215

220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro Gly Ser  
340